Abstract

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Title of Graduation Thesis: Study of HILIC separation mechanism and the influence of mobile phase composition on selectivity and retention of the analytes.

This graduation thesis deals with a study of retention, selectivity and HILIC mechanisms depending on the mobile phase composition. The column Zorbax HILIC plus (2,1 x 100 mm, 3,5 µm, Agilent Technologies), one of the basic types of HILIC stationary phases, was chosen for the purposes of this study. The influence of acetonitrile concentration, buffer pH (3,8, 4,8 and 6,8) and buffer concentration (0,5 – 200 mM) on retention of selected analytes was tested. Based on the physical-chemical properties (log P and pKa) a set of 35 analytes including bases, acids and neutral compounds was chosen. Isocratic flow of 0,7 ml/min and injection volume of 2 µl were used. Detection of analytes was performed by a PDA detector at the wavelength of 254 nm.

The data are presented in charts and tables for an easier evaluation. The effect of concentration of acetonitrile in mobile phase was evaluated by the dependence k´ = f (% ACN). An increasing retention of analytes with increasing concentration of acetonitrile in mobile phase was shown. The effect of pH and buffer concentration on the retention of tested analytes was tested as well. With increasing buffer concentration increasing retention of acid analytes and decreasing retention of basic analytes was observed. Change of mobile phase pH affected especially the retention of basic analytes, increasing pH led to a decrease of their retention. However, lower influence was shown for the acidic analytes, increasing pH led to a slight increase in retention. The buffer pH and buffer concentration effect were insignificant for neutral compounds, nucleosides and nucleobases. There was also studied the effect of pH on selectivity and the results shew, that pH doesn´t affect the selectivity of the tested stationary phase. Lin-log and log-log charts were constructed for the evaluation of retention mechanism. The results confirmed the complex retention mechanism with the predominance of partitioning mechanism.